REMARKS

The examiner has newly rejected claims 1-7, 14, 15 and 25 under 35 U.S.C. §012 as anticipated by the teachings of Walter et al., *International Immunology* 9(3):451-459 (1997). He also has maintained his previous rejection of claims 1-3 under 35 U.S.C. §102 as anticipated by U.S. Patent 6,528,304, issued to Carosella et al. Both references disclose the binding of a specific antibody, W6/32, to a specific recombinant HLA molecule and the subsequent detection of the bound antibody. The examiner has asserted that the antibody is specific to a specific recombinant HLA molecule since the antibody is specific to class I molecules in general as opposed to Class II molecules. These rejections are traversed.

As set forth above, Applicant has amended the pending claims to make clear that the antibodies which are the focus of the claims are ones which specifically recognize a single, particular molecule within a class of MHC (or HLA) molecules rather than simply the class of MHC (or HLA) molecules, respectively, as taught in the cited prior art. The amendments are based on the specification, which make reference to the detection of different alleles. See for example, page 8, line 24, and page 17, from line 1, which refers to the use of different individual recombinant MHC molecules "e.g. relating to one or more epitopes

of a naturally occurring allele." As the cited references only disclose antibodies which recognize a single class of molecule, and cannot further differentiate and recognize a single, particular molecule within the class, the references do not anticipate the cited claims.

The examiner also has rejected claims 1-7, 9-17, 20 and 22-25 under 35 U.S.C. §103, as obvious over U.S. Patent 5,270,169, issued to Chang et al., in view of the Walter et al. reference cited above. The examiner asserted that Chang et al. disclose a method of detecting the presence of anti-HL antibodies by using HLA antigens which may be synthetic. He further asserted that the only difference between this reference and the claims at issue is that Chang et al. do not suggest the use of recombinant HLA antigens but that the use of such an antibody is taught by the secondary reference. This rejection is traversed.

As explained below, Applicants respectfully submit that one of ordinary skill in the art would not be motivated to use recombinant HLA molecules in the method of Chang et al. and that even if there were motivation to use synthetic molecules the motivation would not be for an application in which specific antibodies to distinct MHC alleles would be detected. Additionally, even if one were motivated to combine the teachings

of Chang et al and Walter et al., one would not obtain the method of the present claims.

With regard to the first of these points, as Applicants have noted in responding to a previous Office Action, there has been a resistance in the prior art to the use of recombinant molecules because of their presumed inability to retain their natural epitopic integrity. In particular, it was assumed that the peptide presented in the groove of the MHC/HLA molecule, which could alter the conformation of the MHC/HLA molecule, would affect epitopic integrity. Applicants, however, surprisingly determined that the peptide bound to the MHC/HLA groove is irrelevant during antibody binding. As a consequence, despite previous expectations, it was determined that recombinant MHC/HLA molecules can be used the method of the present invention.

In addition, naturally occurring MHC and HLA molecules are heavily glycosylated. The recombinant molecules of the invention can be synthesized in prokaryotic systems, which does not allow for glycosylation of the protein. Lack of glycosylation of recombinant peptides has been known to alter the conformation of the recombinant protein and alter the ability of antibodies to bind to the protein. As each MHC or HLA molecule has unique glycosylation sites, the position and amount of glycosylation can vary. The Applicants surprisingly discovered that the MHC and

HLA molecules can be synthesized recombinantly in prokaryotic systems, lack glycosylation and still provide an epitope for antibody binding. The Applicants thus have developed technology which went against the current thinking in this field at the time. As such, the use of recombinant molecules in the methods of the present invention is clearly non-obvious and inventive.

In view of the concern in the prior art regarding the use of recombinant antibodies, there is a question as to what Chang et al. were advocating when referring to synthetic HLA antigens. It should be noted in the Example provided by Chang et al. that the method used in that Example is unable to detect different HLA alleles. Instead, the experiment simply determines if <u>any</u> antibodies are present in the sample by binding to HLA antigens obtained from a cultured supernatant of a lymphoblastoid cell line. Thus, clearly one method advocated by Chang et al. is the determination of HLA antibodies in general in a sample, i.e. <u>not</u> allele specific. In performing such methods, retention of the crucial epitopic integrity that distinguishes different HLA alleles is not of paramount importance. In such a case, loss of absolute epitopic integrity can be tolerated. It was believed at the time of the invention that framework components of HLA molecules to which monomorphic antibodies such as W6/32 bind were not as sensitive to disruption as allele-specific epitopes. As

such, the skilled person would possibly consider the use of synthetic MHC molecules if detection of classes of antibodies, rather than allele-specific antibodies was contemplated. The reference in Chang et al. to synthetic HLA molecules thus should be read in this context.

In contrast, it was the view at the time of the invention, as noted above, that the epitopic integrity which uniquely identifies an MHC allele would be lost when produced recombinantly. As such, the use of recombinant molecules in methods such as those described in the present application would not be thought possible using recombinant molecules and, indeed, are not advocated by Chang et al.

The examiner has suggested combining the teachings of Chang et al. with that of Walter et al. Walter et al. teaches that W6/32 binds recombinant HLA antigens. However, this antibody is well known to be directed to a monomorphic region of the antigen present in class I antigens. Retention of this epitope in recombinant molecules would not lead the skilled person to believe that polymorphic epitopes would be retained in recombinant molecules. The W6/32 antibody binds to framework portions of the HLA molecule, whereas allele-specific antibodies bind to epitopes previously thought to be sensitive to recombinant expression. Although Applicants dispute that the

skilled person would seek to combine these documents in the first place, even if he were to do so at best he would find a teaching of the use of general antibodies which can identify a class of HLA molecules in the method of Chang et al. to identify synthetic HLA molecules of a particular class. The secondary reference does not teach that allele-specific antibodies can be detected using recombinant HLA molecules.

The Applicants' work in establishing that recombinant molecules actually could be used to identify allele-specific antibodies and that the effects of glycosylation and the use of non-specific peptides did not lead to a loss of relevant allele-specific epitopic sites has allowed, for the first time, the development of a method of detecting specific MHC antibodies which was not previously possible. Although with hindsight it may seem that there were pointers in the prior art, that simply is not the case, and the Applicants overcame a conceptual hurdle which had blocked the use of recombinant molecules for applications relying on the retention of specific epitopic integrity. In doing so, they have for the first time provided a method which is able to detect specific antibodies to specific MHC alleles which simply was not possible with earlier techniques which used isolated MHC molecules which contained pools of antigens which would consist of more than a single allele.

Finally, claims 24-28 have been rejected under 35 U.S.C. §103 as obvious over the teachings of Chang et al. in view of Walter et al. and further in view of a reference by Luxembourg et al. For the reasons set forth above in relation to the patentability of the claims from which these claims depend, Applicants submit that the primary and secondary cited references do not make obvious the detection of allele-specific antibodies using recombinant MHC molecules. The addition of the teachings of Luxembourg et al. does not overcome the deficiencies of the other references.

The recombinant MHC molecules that were generated were suggested for use in T-cell antigen receptor recognition via the peptide rather than by recognition of unique epitopes of the MHC molecule. Therefore, the best that Luxembourg et al. would seem to show is that recombinant MHC antigens are suitable for peptide presentation. They do not, however, show that epitopic sites which discriminate different MHC alleles could be retained. As Applicants have argued in previous submissions, T cells utilize a different epitope on the MHC or HLA molecules in comparison to antibodies, and thus T-cell binding to recombinant molecules is not illustrative of any utility of recombinant molecules for antibody binding.

Luxembourg et al. teach nothing about the use of recombinant molecules for the isolation of allele-specific anti-MHC antibodies. The examiner indicates that the reference teaches the use of the system for the isolation of peptides such as antibodies. The passage to which the examiner refers, however, is merely concerned with quantifying the number of MHC molecules immobilized per bead. The ability of recombinant molecules to bind to naturally occurring antibodies--and more particularly whether they would be able to be discriminated by allele-specific antibodies--is simply not addressed. As such, none of the documents, considered alone or in combination, provides the relevant teaching to make obvious the claims of the present application.

Applicants respectfully submit that in view of the amendments and discussion set forth above, the pending claims of the application now are in condition for allowance.

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